Developing a Noninvasive Tracking Method using Click Chemistry to study Novel Cancer Immunotherapy

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Introduction

- Recent developments in cancer immunotherapies, such as CAR T cell therapies, have shown great success. However, these therapies require diagnostic tools, such as cell tracking methods, to reveal their mechanism.
- We focused on developing a cell tracking method that directly labels the cell surface with an alkyne, which can be reacted with a radiolabeled azide via a Cu-catalyzed click reaction **(Figure 1A)** and visualized through PET/CT.
- To utilize this imaging method, a Cu-chelating azide must be developed in order to reduce cytotoxicity from exposure to Cu(I) and, ultimately, generate a single isolable reagent for *in vivo* administration **(Figure 1B)**.
- The Farwell Lab has synthesized multiple Cu-chelating azides and tested their ability to react with alkynes in a setting without cells.
- However, the Cu-chelating azide's ability to react with cell surface alkynes and reaction kinetics *in vitro* require further testing
- **Aim**: develop an *in vitro* kinetic assay to test the reaction rate of the Cu-chelating azide

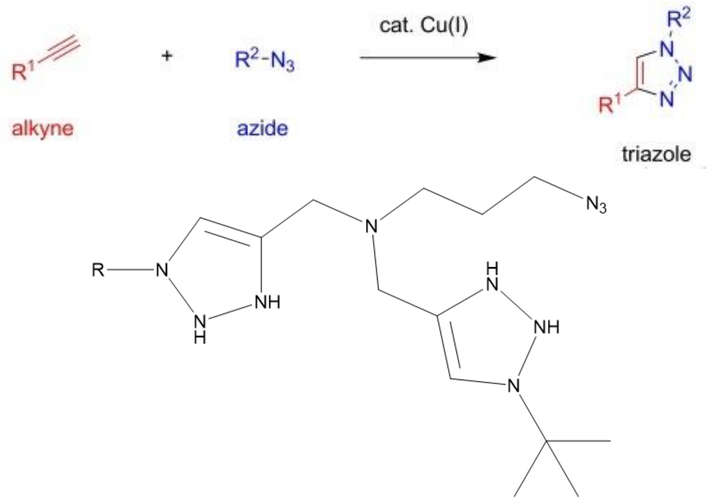
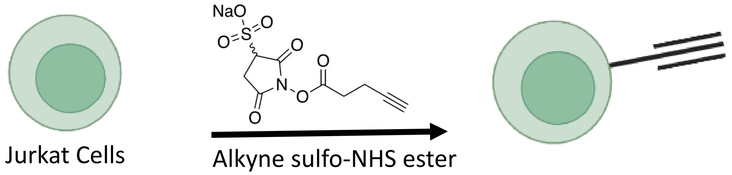


Figure 1: (A) Cu-catalyzed click reaction. **(B)** Representative Cu-chelating azide with two triazole groups. The R group can contain a radiolabel, such as ^{18}F, or a fluorophore depending on desired use.

Methods

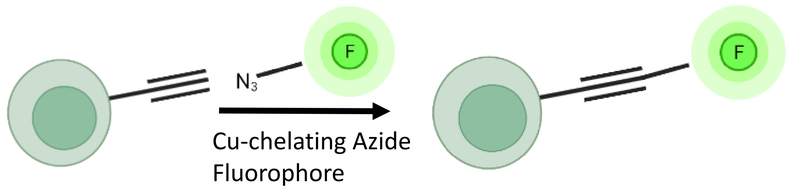
Standard Workflow to react Surface Alkyne with a Cu-chelating Azide Fluorophore

1. Label the Cell Surface with Alkyne



2. Wash excess Alkyne 2x

3. React Surface Alkyne with Azide

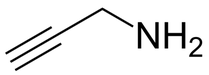


4. Wash excess Fluorophore 3x

5. Measure the Fluorescence via Flow Cytometry

Quench Test for the Cu-chelating Azide

The quench test follows the standard workflow. However, prior to incubation with the Cu-chelating azide fluorophore, propargyl amine, an alkyne reagent able to react with azides, was added at various concentrations: 2, 5, and 10 mM.



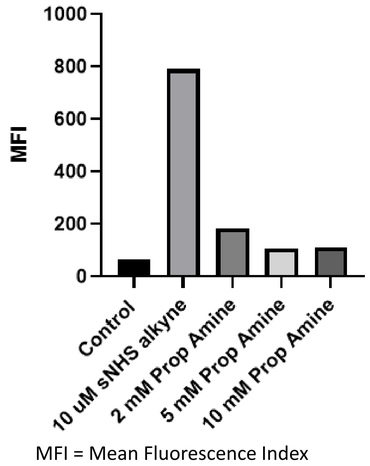
Propargyl Amine

In vitro Kinetic Assay for Cu-chelating azide

The kinetic assay differs from the standard workflow by varying the amount of time that is allowed for the surface alkyne to react with the Cu-chelating azide. Jurkat cells are first incubated with 5 µm sulfo-NHS ester alkyne. After incubating with a 10x excess of Cu-chelating azide (50 uM) for 2, 5, 10, 20, and 25 minutes, respectively, the reaction was quenched with 5 mM Propargyl Amine. The data can then be analyzed using GraphPad Prism to fit an exponential based on a pseudo first-order reaction to calculate the rate constant.

Results and Discussion

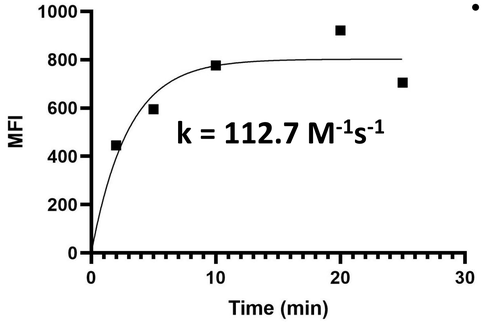
Quench Test for the Cu-chelating Azide



MFI = Mean Fluorescence Index

- Higher fluorescence indicates that the click reaction between the Cu-chelating azide and the cell surface alkyne has occurred to a greater degree.
- There was successful quenching of the click reaction at high concentrations of propargyl amine (>5 mM) shown by near-control levels of fluorescence
- Identifying successful quench conditions allowed us to perform the kinetic assay with accurate measurement of incubation time

Kinetic Test for Cu-chelating Azide



- Longer incubation times with the Cu-chelating azide allowed the click reaction to approach completion
- Pseudo first-order rate constant k=112.7 $M^{-1}s^{-1}$
- With Cu-chelation, the rate of reaction was more than ten times faster than a Cu-catalyzed reaction without Cu-chelation (k ~ 10 $M^{-1}s^{-1}$)

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